CHAPTER 4

Conclusions

An analysis method for two aliphatic amines, dimethylamine (DMA) and trimethylamine (TMA) used for seafood, fish and shrimp, freshness indication was investigated using headspace gas chromatography with nitrogen phosphorous detector (HS-GC-NPD). Chromatographic separation was carrier out by a fuse silica HP-FFAP capillary column, a 25 m × 0.32 mm i.d. × 0.52 μm film thickness consists of nitroterephthalic acid modified polyethylene glycol. Optimum conditions for GC-NPD technique were; flow rate *i.e.*, carrier gas (helium gas) 3 mL min^{-1}, fuel (hydrogen gas) 2 mL min^{-1}, oxidant gas (air) 100 mL min^{-1}; injector and detector temperature 120°C and 220°C, respectively; split ratio 10:1; column temperature programming 120°C followed by an increasing to 160°C with the temperature ramp rate of 30°C min^{-1} and kept at final temperature for 1 min.

In headspace system, a laboratory-built stainless steel water bath with a control heating coil and stainless steel adapter for vial holder was optimized and the results obtained are equilibration times 20 min, equilibration temperature 70°C, phase ratio 2.0. Vial volume at 10 mL was used to prepare sample to decrease the amount of sample. In addition, 1.5 g of Na_2CO_3 was added to increase the sensitivity of both analytes and decrease the solubility of analytes in matrix (Kolb and Ettre, 1997).

At optimum conditions good resolution ($R \geq 1$) and short analysis time (<5 min) were obtained. The HS-GC-NPD system showed a wide linear dynamic range, 2.7×10^{-3}-250 μg mL^{-1} for DMA and 0.30×10^{-3}-50 μg mL^{-1} for TMA with a coefficient of determination (R^2) greater than 0.99. The limit of detection was 2.7 ng mL^{-1} for DMA and 0.30 ng mL^{-1} for TMA. Excellent precision was obtained since the relative standard deviations (RSD) were all lower than 4%.

The matrix interference was also studied. Statistically fish and shrimp samples interfered with the responses and matrix match calibration curve was used for determination of DMA and TMA in the sample. The method was validated to ensure the reliability of the results by using standard, spiked samples, reagent and method

blanks. Validation parameters include accuracy, recovery and precision were studied. Recoveries were obtained at 54-69% for DMA (0.5 and 1 μg mL^{-1}) and 53-70% for TMA (0.5 and 1 μg mL^{-1}). For DMA and TMA there are no regulation for recovery but a recovery between 13 and 109% are acceptable by EPA method 8070A in 1996 for the analysis of nitrosamines by gas chromatographic method in solid matrices which are similar to DMA and TMA. Relative standard deviations (RSD) were 3.8-11.8% (n=5), lower than 20% (EPA, 8021B), the maximum %RSD that is allowed at spiked concentration 100 and 200 μg mL^{-1} for DMA and 0.5 and 1 μg mL^{-1} for TMA. When comparing between HS-GC-NPD method of this work with other reports (Table 4.1) it can be seen that the sample size, 1.5 g is smaller than almost all other methods other advantages of this HS technique includes being solvent free, simple to operate with less procedure steps and donot required derivatization. In term of system performance, the detection limit of this work (3.0×10^{-4} mg 100 g^{-1}) was better than others (Sukpeng, 2001; Vciana-Nogues *et al.*, 1996; Li *et al.*1997; Namieśnik *et al.*, 2003; Kaykhaii *et al.*, 2005). The recovery in this study (53-70%) is lower than other work, however, they all has the preconcentration steps, *i.e.*, by LLE (Vciana-Nogues *et al.*, 1996), SPME (Li *et al.*, 1997). Aliphatic amines with SIBA were also used for derivatization coupled to HS-SPME (Zhao *et al.*, 2003) and HS-SDME (Kaykhaii *et al.*, 2005) for liquid samples, making it easier to extract than solid samples, hence, higher recovery.

Table 4.1 Comparison between proposed method and another sample preparation method for analysis aliphatic amines in seafood

Samples	Amount of sample	Pre-treatment	Recovery (%)	Detection limits (mg 100 g^{-1})	References
Fish and shrimp	1.5 g	HS	53-70	3.0×10^{-4}	This work
Fish	50 g	HS (based on air sampling with Cabotrab or Tenax sorbent tubes) , LLE	No reported	No reported	Krzymien and Elias, 1990
Fish	10 g	LLE	97-99	0.46	Vciana-Nogues *et al.*, 1996
Fish	1 g	SPME	93	7.5×10^{-3}	Li *et al.*, 1997
Frozen seafood	20 g	HS (based on air sampling with silica gel sorbent tubes)	No reported	0.48	Sukpeng, 2001
Lake water	100 mL	Derivatization with SIBA and HS-SPME	94-102	6.4×10^{-5}	Zhao *et al.*, 2003
Air	3 g	SPME	No reported	0.67×10^{-4}	Namieśnik *et al.*, 2003
Tap and river water	2 mL	HS-SDME	96-103	2.5×10^{-4}	Kaykhaii *et al.*, 2005

For qualitative and quantitative analysis of DMA and TMA in fish and shrimp, these samples were analysed and evaluated using the matrix match calibration curve. The concentrations of DMA in all samples were obtained in the range of 2.17-5.15 mg per100g, TMA in fish and shrimp samples were obtained in the range ND-7.69 mg per 100g. These concentrations of DMA and TMA in fish and shrimp samples were lower than the EU acceptable quality and safety limit (TMA, 12 mg per 100 g) (The European Commission Council Regulation No. 91/493/EEC, 1991). For DMA, although there is no specific limit but it can react with nitrile compounds to form a nitroso-dimethylamine (carcinogen compound).

In conclusion, the proposed analysis method can be used for qualitative and quantitative analysis of DMA and TMA in fish and shrimp samples with good recovery and precision. This method has many advantages such as simplicity, less procedure *versus* LLE (Vciana-Nogues *et al.*, 1996) and derivatization technique (Zhao *et al.*, 2003); rapid, the throughput of the analysis is 12 samples per hour *versus* 4 sample per hour (Sukpeng, 2001)); small sample size (1.5 g *versus* 20 g for HS technique (Sukpeng, 2001); solvent free, no solvent *versus* 1 mL toluene per tube (Vciana-Nogues *et al.*, 1996); low detection limit in HS technique, 2.7×10^{-4} mg per 100 g *versus* 0.48 mg per 100 g (Sukpeng, 2001); cost effective, 1 bath (1.5 g sample)/vial and can be tested freshness of seafood.